THE AMINO ACID REQUIREMENTS OF MAN

BY

MINOR J. COON

B. A., University of Colorado, 1943

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE SCHOOL OF THE
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ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Professor Wm. C. Rose, who suggested these research problems and in directing their investigation was a constant source of inspiration.

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THE AMINO ACID REQUIREMENTS OF MAN

I. GENERAL INTRODUCTION

Inasmuch as the question of the amino acid requirements of man is of primary interest to medical science and nutrition, many attempts have been made to estimate the needs of the adult human organism. In 1942 an experimental approach to the problem was undertaken in this laboratory. Nitrogen equilibrium studies (1,2,3) were carried out on young men ingesting diets containing mixtures of highly purified amino acids which supplied over ninety-five per cent of the nitrogen intake. The amino acids essential for man were found to be tryptophane, phenylalanine, lysine, threonine, valine, leucine, isoleucine, and methionine. The quantitative requirements for the first five of these have also been investigated (4,5,6,7).

In the present study, further data on the requirements of the human organism for phenylalanine and lysine were accumulated. Experiments dealing with the possible utilization of acetyl-*D*-tryptophane* and with the caloric requirements for the maintenance of nitrogen equilibrium also were carried out.

II. THE QUANTITATIVE PHENYLALANINE REQUIREMENT OF MAN

Introduction

From the results of a recent study of man's requirement for phenylalanine (5) it was concluded that the daily intake for the maintenance of nitrogen equilibrium need not exceed 1.87 gm. The present work was carried out in order to investigate this problem further, and to study the utilization of the *D* isomer of the amino acid.

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*The notations *D* and *L* are used with reference to the configuration on the α-carbon of the amino acids.

Experimental

Three male graduate students served as subjects. Their rations consisted of amino acids, urea, corn starch, sucrose, butter, corn oil, lemon juice, Cellu flour, minerals, haliver oil concentrate, and a mixture of vitamins. The ratio of carbohydrate to fat calories, 2.6, and the total daily caloric intake were kept constant throughout the experiment. Distilled water was taken *ad libitum*. Twenty-four hour samples of urine were collected and preserved with toluene, and aliquots were analyzed for creatinine and total nitrogen. Fecal periods were marked off with charcoal tablets, and the total nitrogen content was determined. After the subject had attained positive nitrogen balance on a diet adequate in the essential amino acids, his intake of phenylalanine was lowered in successive experimental periods. The total nitrogen intake was kept constant, however, by adjusting the amount of glycine consumed. In this manner a level of phenylalanine could be determined which was sufficient to maintain the subject in positive nitrogen balance, although an intake of 0.1 gm. less led to negative balance, or to a fluctuation between positive and negative balances.

Summary

Of the three individuals studied, two required 1.1 gm., and the third required 0.9 gm. of *L*-phenylalanine per day.

When the *dl* modification was fed, it appeared that about three-fourths of the *D* isomer was utilized. When the *D* form was administered alone the utilization seemed to be somewhat less. Additional experiments will be required in order to establish accurately the extent to which *D*-phenylalanine can replace the *L* isomer.

III. THE QUANTITATIVE LYSINE REQUIREMENT OF MAN

Introduction

In a previous investigation (6) it was found that 0.4 gm.

of *L*-lysine was a sufficient daily intake for the maintenance of nitrogen equilibrium in each of two human subjects, whereas 0.3 gm. was inadequate. In subsequent experiments on other individuals the amino acid was administered at the level of 0.6 gm. It soon became evident, however, that certain individuals are unable to maintain consistent positive nitrogen balance until the intake of the amino acid is increased. In the present investigation the lysine requirements of three subjects of the latter group were determined.

Experimental

The minimum level of lysine for each subject was established by a procedure similar to that described above for phenylalanine.

Summary

Two of the subjects studied required 0.8 gm. of *L*-lysine per day, and the third required 0.7 gm.

IV. THE AVAILABILITY OF ACETYL-*D*-TRYPTOPHANE IN MAN

Introduction

Since the human species does not utilize *D*-tryptophane to any large extent, if at all (4), one would hardly predict that man would have the ability to utilize the acetyl derivative of this isomer. Nevertheless such a report has come from the laboratory of Albanese and his associates (8), based on an estimation of the urinary excretion of tryptophane and of an unidentified indole metabolite which they had previously described (9). An attempt was made to confirm by a nitrogen equilibrium study the conclusion reached by these workers.

Experimental

The two graduate students who served as subjects exhibited consistent positive nitrogen balance on an intake of 0.20 gm. of *L*-tryptophane per day. However, when this

amino acid was replaced by an equimolar amount (0.24 gm.) of acetyl-*D*-tryptophane, consistent negative nitrogen balance ensued.

Summary

By nitrogen equilibrium studies on two human subjects it was shown that neither is able to utilize acetyl-*D*-tryptophane in place of an equivalent amount of *L*-tryptophane.

V. A COMPARISON OF PROTEIN, PROTEIN HYDROLYSATES, AND AMINO ACID MIXTURES FOR THE MAINTENANCE OF NITROGEN EQUILIBRIUM IN MAN

Introduction

From the beginning of the investigation of the amino acid requirements of man in this laboratory, it has been noted that high caloric intakes are necessary to bring adult human subjects into positive nitrogen balance. Many individuals require for this purpose about 55 calories per kilo of body weight, or somewhat over 2,000 calories per square meter of surface area. The question therefore arose as to whether a high energy requirement is peculiar to the ingestion of greatly purified amino acids.

Experimental

A study was made of nitrogen equilibrium in subjects receiving whole protein, acid- or enzyme-hydrolyzed protein, or mixtures of individual amino acids as a source of nitrogen, along with various levels of caloric intake. Acid-hydrolyzed casein was prepared according to the directions of Berg and Rose (10), and the product was supplemented with *L*-tryptophane. Amigen was used as an enzymatic hydrolysate of casein. Of the amino acid mixtures used in the study, some were designed to provide the eight essential acids (with additional nitrogen furnished in the form of glycine and

urea), and others were formulated so as to imitate the complete composition of casein.

SUMMARY

Judging by the magnitude of the energy intake required to effect positive nitrogen balance in man, it appears that the supplements tested have the following decreasing order of effectiveness: whole casein, acid- or enzyme-hydrolyzed casein, purified amino acid mixtures.

Recently Womack and Rose (11) reported evidence for the existence of an unidentified component in proteins which stimulates growth in rats. In the case of mice, as well as of certain bacteria, Woolley (12) has found that growth is also promoted by certain whole proteins. These findings are extremely suggestive of the results obtained in the present study with human subjects. Possibly purified casein contains an unknown factor which adult man requires in order to maintain nitrogen equilibrium upon a normal caloric intake. Further experiments will be necessary before a definite decision can be reached in this important problem.

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VITA

The author was born July 29, 1921, in Englewood, Colorado. He received his elementary and secondary education in the Denver public schools. He attended the University of Colorado, from which he received the Bachelor of Arts degree, with a major in chemistry, in June of 1943. He entered the graduate school of the University of Illinois in September, 1943, as a half-time Special Research Assistant in Chemistry. He received this appointment on a full-time basis in June of 1944, and held it for the two succeeding years.